Iron Deficiency and Neurodevelopment - Potential Targets for Intervention from Pre-Clinical Models

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Driven to Discover℠

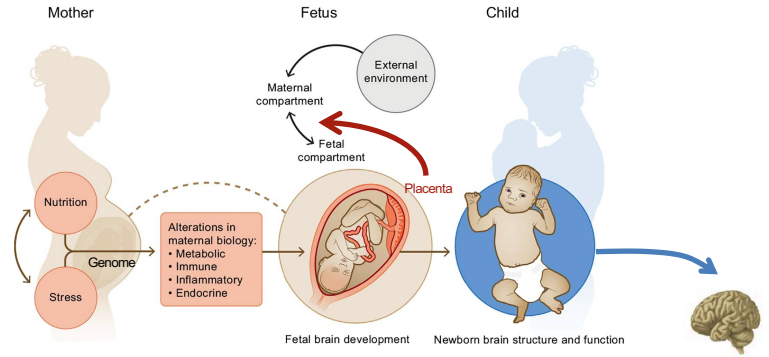
Disclosure Statement

- I have no financial relationships to disclose or Conflicts of Interest (COIs) to resolve.

Overview

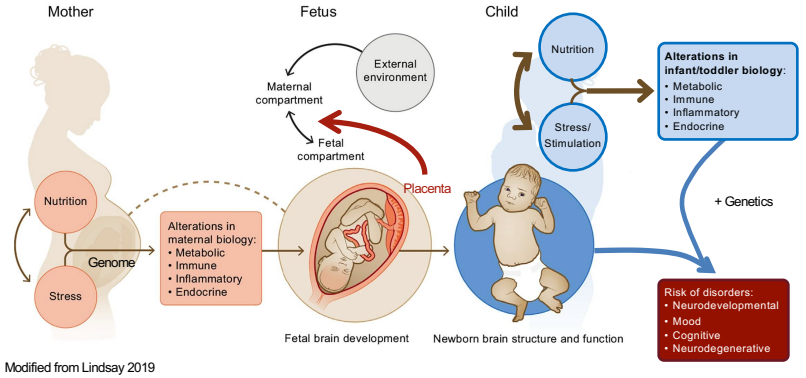
- Nutrition and brain development
- Early-life iron deficiency (ID)
  - Causes
  - Effects on long-term neurobehavioral function
- Neuron culture model of early-life ID
  - Potential mechanisms
  - Interventions

How is the Brain Built?



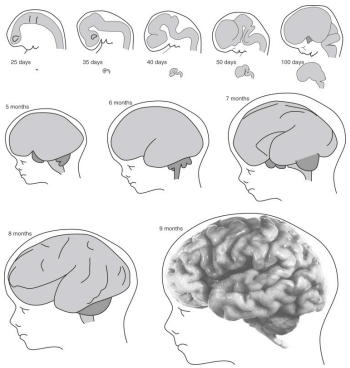
Modified from Lindsay 2019

How is the Brain Built?

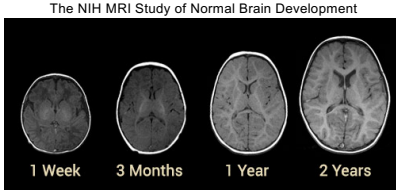


Modified from Lindsay 2019

Early Brain Development



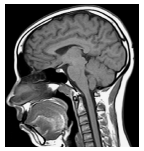
The NIH MRI Study of Normal Brain Development



Brain development is energetically demanding

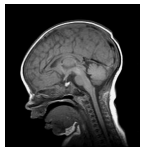
Rapp and Bachevalier 2013; Kuzawa 1998

High Energetic Needs of Brain Development



Adult

- Need ~40 kcal/kg/day
  - 20% of energy intake goes to the brain



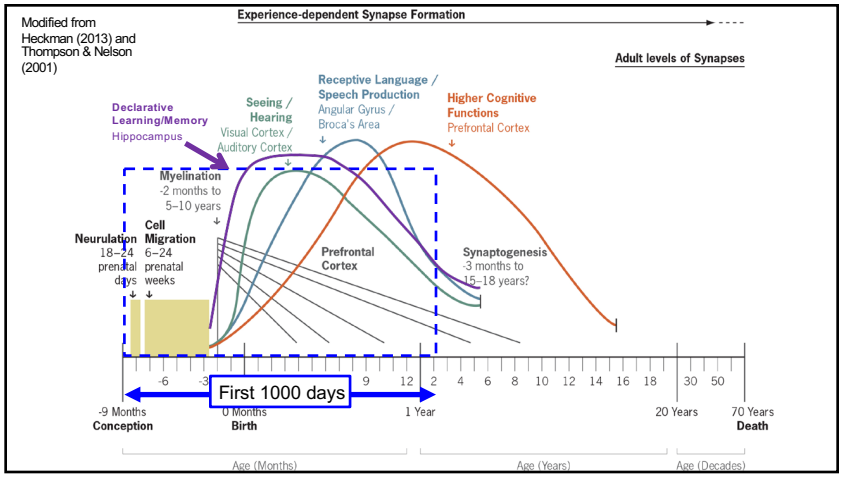
Newborn

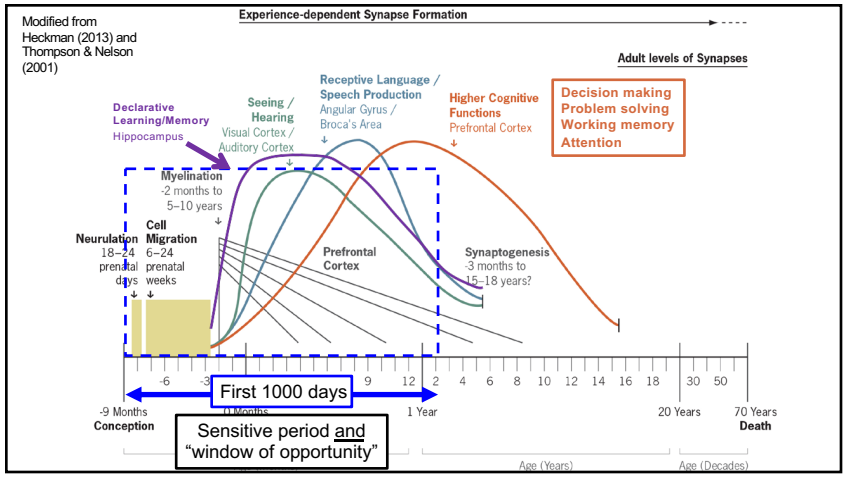
- Need ~110 kcal/kg/day
  - 60% of energy intake goes to the brain!

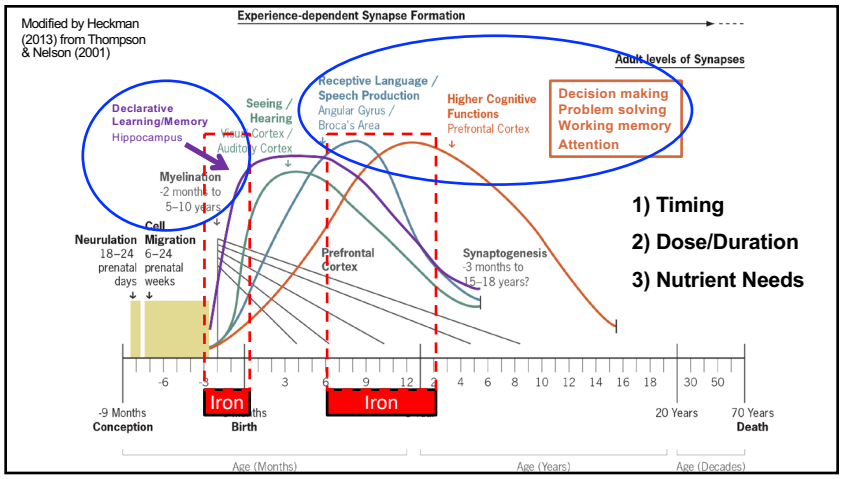
Kuzawa 1998; Devi 2015; Consolini 2016

Nutrition and Brain Development

- All nutrients are necessary for brain development during both prenatal and early postnatal life
- **Metabolic Substrates** – Nutrients or molecules that support cellular energy metabolism (i.e., ATP production)
  - Macronutrients (e.g., protein, fats, glucose)
  - Oxygen
  - Micronutrients (e.g., **iron**, iodine, zinc, copper)
  - Other Vitamins/Cofactors (e.g., Vitamins B6 and B12, folate and choline)





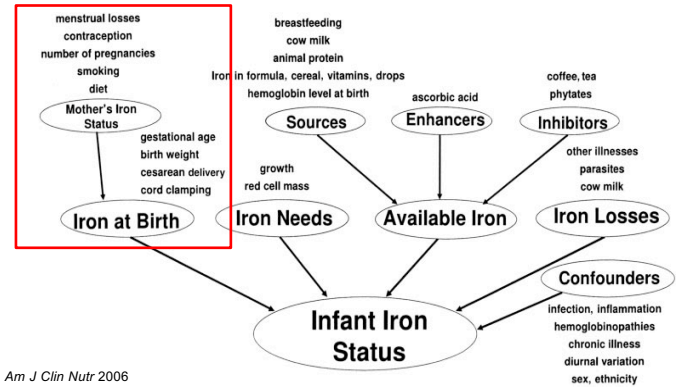


Early-Life Iron Deficiency

- Iron deficiency (ID) with or without anemia is the most common micronutrient deficiency – 2 billion people
  - 50-80% of pregnant women and children in low-resource countries
  - 15-42% of pregnant women/children in the U.S.
- Fetus, infant, toddler are most susceptible to brain ID and neurobehavioral dysfunction
- Causes:
  - Decreased iron supply
  - Increased iron demand (tissue iron redistribution)

Yip 1994; Walker 2007; Mclean 2009; Cogswell 2009; Mei 2011; Auerbach 2019

Factors Determining Infant Iron Status

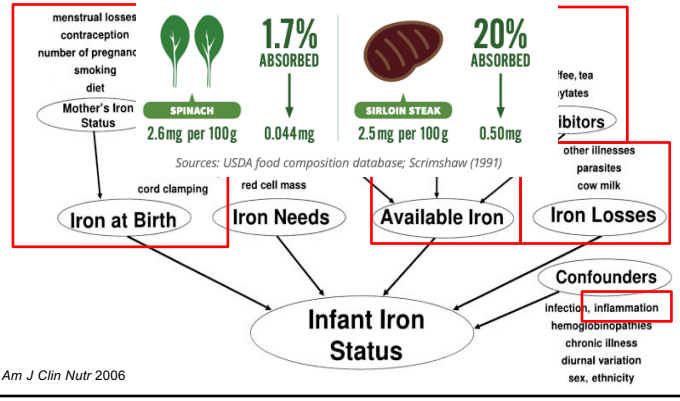


Lozoff, *Am J Clin Nutr* 2006

Decreased Fetal-Neonatal Iron Supply

- Maternal IDA
  - Fetal iron status affected when maternal Hgb <85 g/L or serum ferritin <14 µg/L (Bora 2014)
- Placental insufficiency due to maternal hypertension (IUGR)
  - 32% reduction in brain iron (Georgieff 1995)
- Prematurity
  - 60-70% of maternal-fetal iron transfer is during 3rd trimester (Bothwell 2000)
  - Frequent blood draws (phlebotomy-induced anemia)

Factors Determining Infant Iron Status



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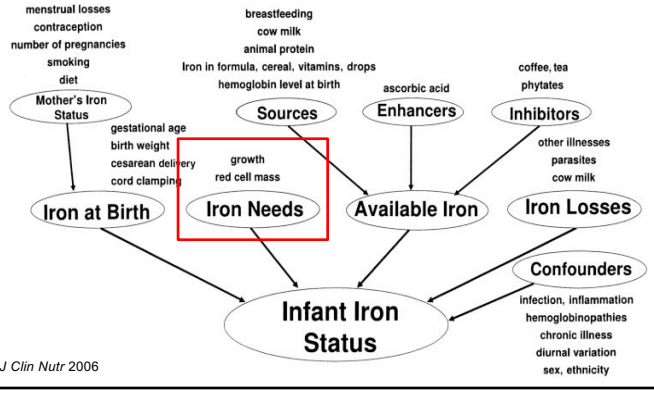

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Increased Iron Demand

- **Iron is prioritized to RBCs over the fetal-neonatal brain when iron demand is greater than iron supply**
  (Petry 1992; Zamora 2016; Rao 2016; Ennis 2018)
- Gestational diabetes
  - Fetuses of diabetic mothers have increased RBC production due to hyperglycemia causing low blood oxygen levels
  - Iron is redistributed from tissues to support RBC production
    - Liver > Skeletal Muscle > Heart > Brain
    - ~40% reduction in brain iron (Petry 1992)
- Prematurity and IUGR
  - Catch up growth

Iron and Brain Development

- Iron-containing enzymes and proteins are involved in cellular processes important for the developing brain:
  - **Axon Myelination** - Speed of processing
    - Lipid and ATP production in oligodendrocytes
  - **Neuronal Structural Complexity –** Neural circuit formation (e.g., learning/memory)
    - Neuronal and glial ATP production
  - **Monoamine Neurotransmitters** - Reward, mood, memory, motivation, motor control, and alertness
    - Tyrosine and Tryptophan hydroxylase synthesis of dopamine, serotonin, norepinephrine

ID Impairs Brain Function in Neonates, Infants, and Toddlers

Long-Term Neurobehavioral Effects of Early-Life ID

Long-Term Neurobehavioral Effects of Early-Life ID in Humans

- Fetal-Neonatal ID
  - Worse retrieval **memory** at 3.5y in infants of diabetic mothers (Riggins 2009)
  - Impaired **language**, fine **motor**, and **attention** at 5y (Tamura et al 2002)
  - Increased risk of **schizophrenia** in adulthood with maternal IDA (Insel et al 2008)

Long-Term Neurological Effects of Early-Life ID in Humans

- Infant-Toddler ID
  - Impaired **motor** function throughout childhood (Shafir 2006)
  - Slower auditory and visual **processing speed** at 4y (Algarin 2003)
  - Increased risk of **ADHD** at 5y (Doom 2014)
  - Worse inhibitory control and recognition **memory** at 10y (Algarin 2013; Congdon 2012)
  - Increased risk of **depression** and **anxiety** in adolescence (Lozoff 2000)
  - Impaired executive functions, recognition **memory** and cognitive test scores at 19yr (Lukowski 2010; Lozoff 2006)
  - Altered **brain connectivity** at 21y (Algarin 2017)

Iron and Brain Development

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Summary of ID and Neurodevelopment – Pre-Clinical Animal Models

- Fetal-neonatal and postnatal IDA in rodents mimic the corresponding human condition
  - Similar degree of brain ID
- Long-term dysfunction in hippocampus, striatum, cortex and cerebellum and behaviors
  - Learning/memory, social interaction, motor impairments and increased anxiety
  - Impaired metabolite concentrations, gene expression, myelination, dopamine neurotransmission, dendrite structure, synaptic function
- Recovery depends on timing and dose of ID and iron treatment
- Neuronal ID without anemia causes similar neurodevelopmental and long-term deficits

Clinical Problem and Management

- Problem: Long-term cognitive and psychosocial deficits and increased risk for adult mood disorders
- Prevention:
  - Better to build the brain right the first time
- Early Diagnosis:
  - Current (AAP) – Screen for anemia at 12mo
    - Late in postnatal iron-sensitive period and brain ID occurs before onset of anemia
  - Not great biomarkers of ID
    - Current biomarkers can be altered by inflammation and other non-ID conditions
    - Need biomarkers of brain ID prior to anemia (Raghu Rao, Michael Georgieff)
- Treatment:
  - Just give back iron
  - Adjunct therapies?

Basic Principles

- Early-life iron deficiency (ID), with or without anemia (IDA), causes long-term neurobehavioral dysfunction
- Anemia is the end-stage of early-life ID
  - Iron is prioritized to RBCs over other organs
  - Brain ID occurs before onset of anemia
- It is neuronal ID, independent of anemia, that is responsible for the persistent structural and learning/memory deficits
- Current diagnosis/iron repletion strategies are not-sufficient
  - Early detection and repletion is key
- Are these kids just out of luck or could we provide treatment in addition to or instead of iron therapy?
  - Adjunct therapies that target the underlying iron neurobiology
    - Better definitions of iron-sensitive critical period

Potential Mechanisms of Long-Term Learning and Memory Deficits

1. Persistent impairment in expression patterns of synaptic genes
   - Epigenetic modifications (e.g., methylation)

Choline

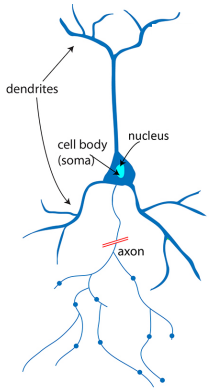
2. Residual neuron structural deficits
   - Not enough iron during energy-demanding critical period of hippocampal neuron maturation

Neuron Structure

- Dendritic arborization
- Dendritic spine head maturation
- Axonal growth and branching

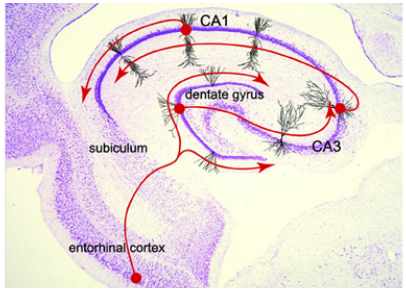
↓

- Synapse formation

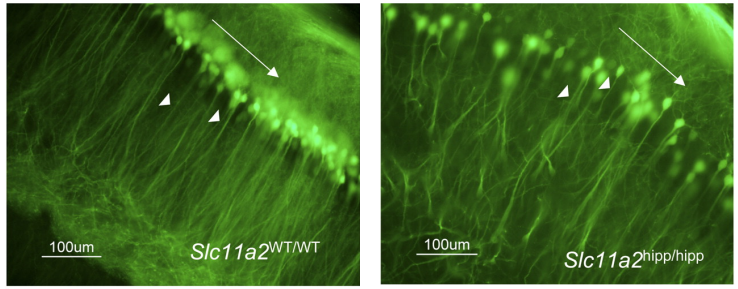


Hippocampal Neuron Structure – Learning and Memory

- Hippocampus mediates recognition memory of
  - Facts, objects and events
  - Spatial mapping
- Learning and memory circuit formation/function depends on proper structural development of hippocampal neurons

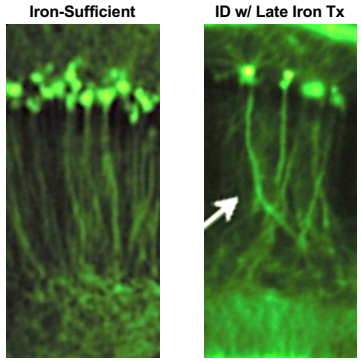


Early-Life ID and Adult Neuron Structure and Learning/Memory



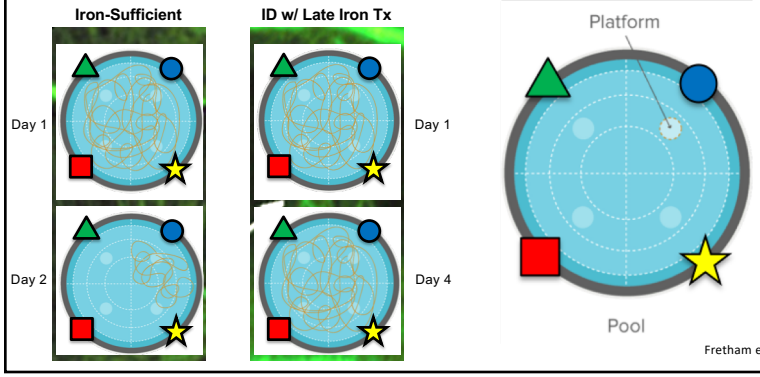
Carlson et al., 2009

Early-Life ID and Adult Neuron Structure and Learning/Memory



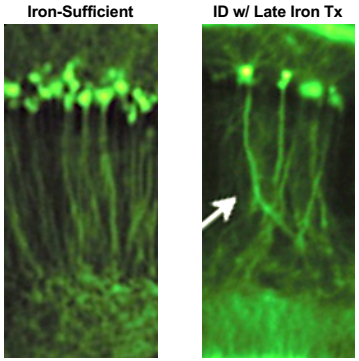
Fretham et al., 2012

Early-Life ID and Adult Neuron Structure and Learning/Memory



Fretham et al., 2012

Early-Life ID and Adult Neuron Structure and Learning/Memory



Hippocampal dendritic complexity and spatial learning/memory are not rescued by late iron repletion following early-life neuronal ID

Fretham et al., 2012

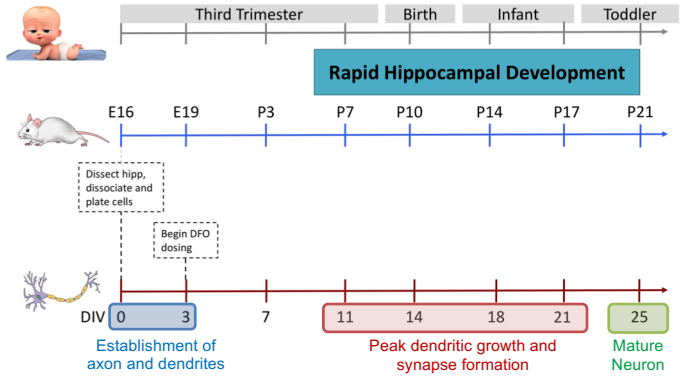
Research Objectives

1. Understand the cellular/molecular mechanisms driving the neuronal structural deficits that impair long-term learning and memory
2. Uncover novel targets for intervention, beyond simply giving back iron, in order to prevent or rescue learning and memory deficits

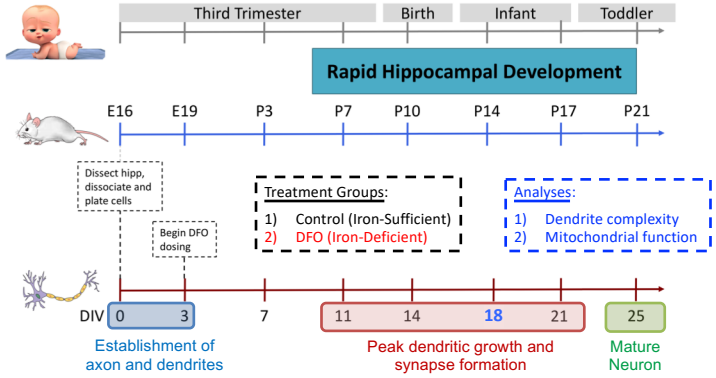
Approach

- Develop a primary hippocampal neuron culture model of ID that mimics the *in vivo* condition during the iron-sensitive period of rapid neuron development

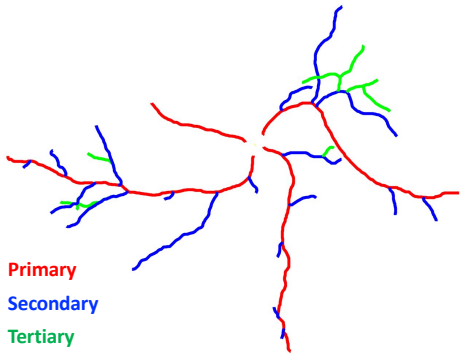
Neuronal ID Culture Model



Neuronal ID Culture Model



Neuronal Dendrite Complexity Analysis



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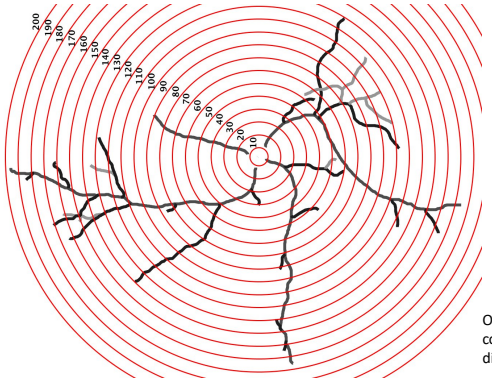
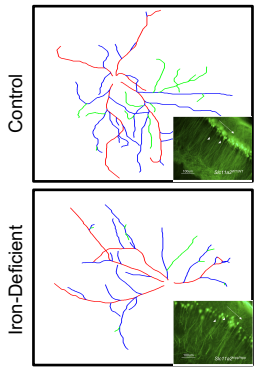
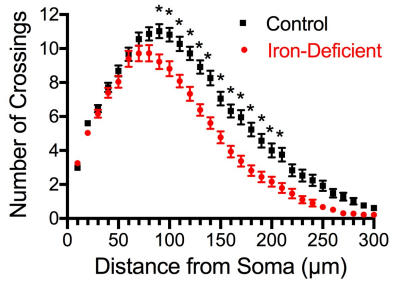


Overall dendritic complexity relative to distance from soma

ID Reduces Overall Dendrite Complexity



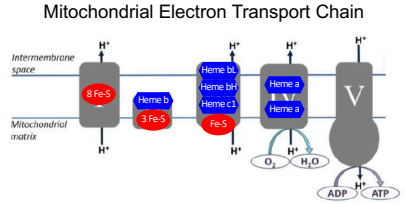


* $p < 0.05$, n=72-90 neurons

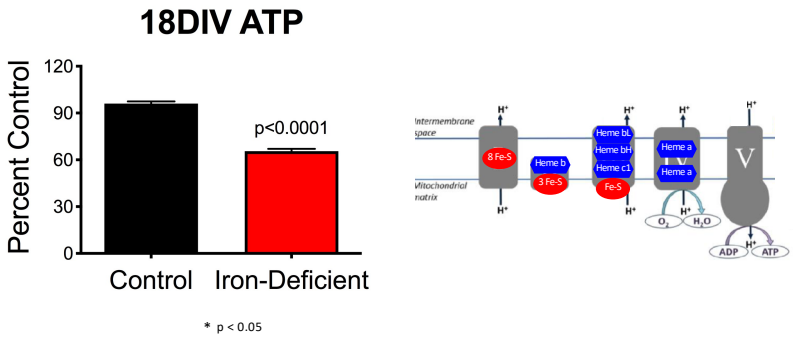
Bastian et al., 2016

Mitochondria and Dendrite Development

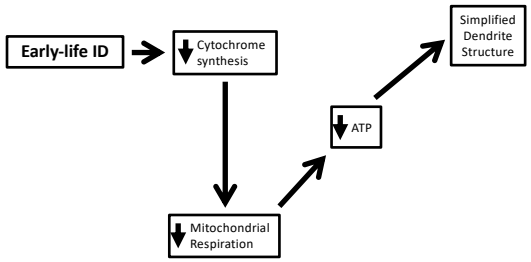
- Dendrite growth is ATP-dependent
- Mitochondrial respiration is iron-dependent



Neuronal Mitochondrial Respiration

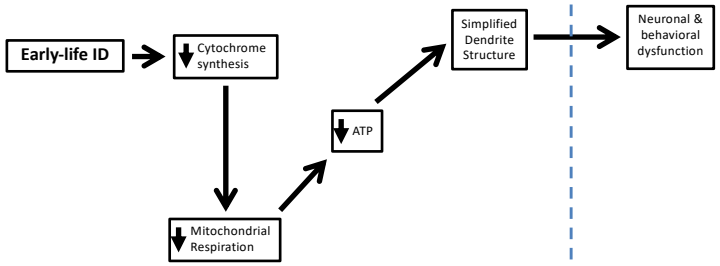


Part 1 – Summary and Working Model

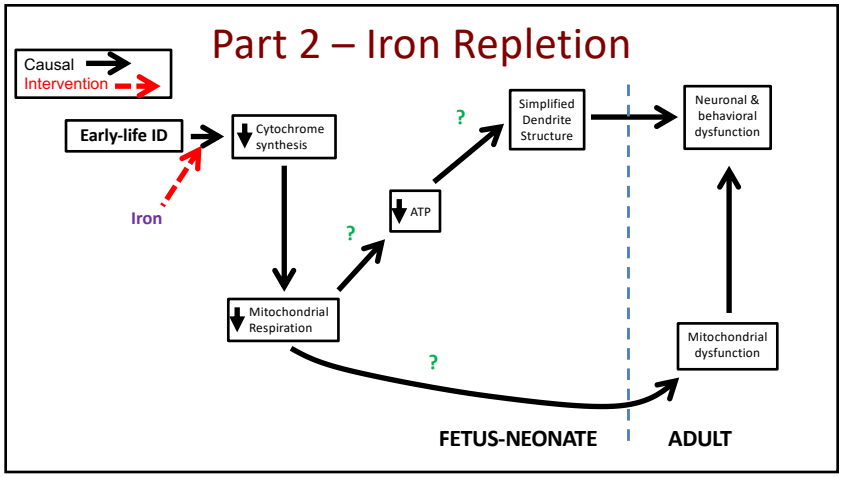


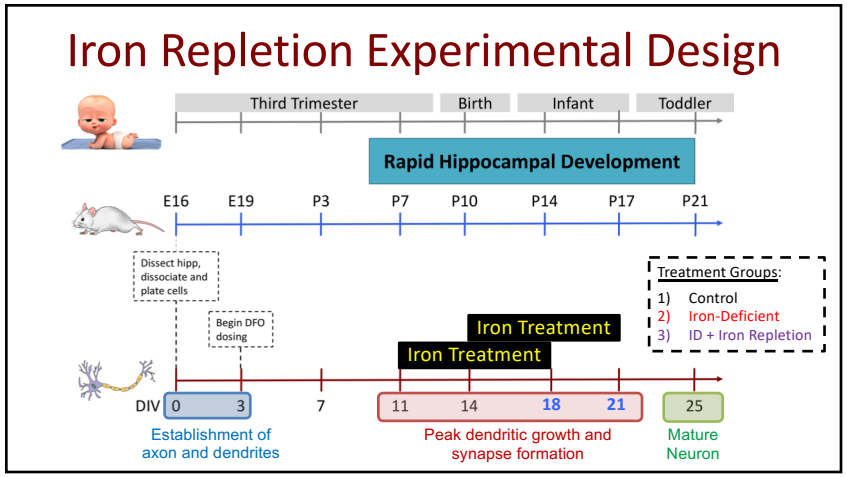
FETUS-NEONATE

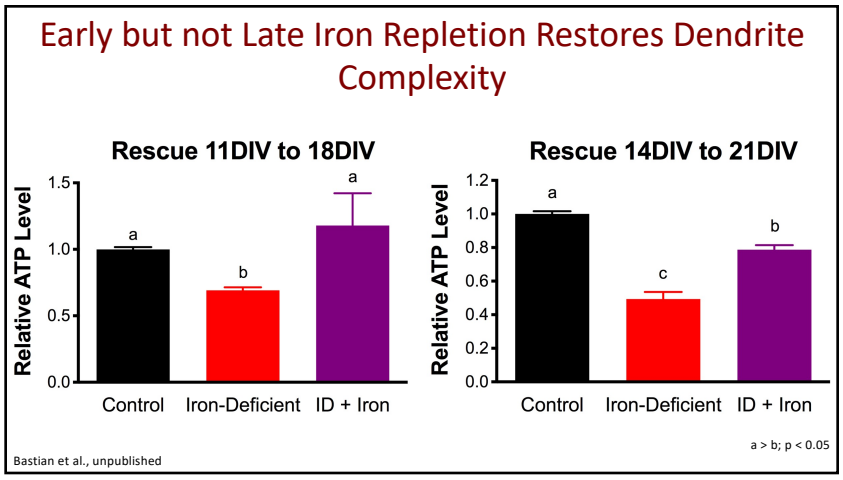
Part 1 – Summary and Working Model

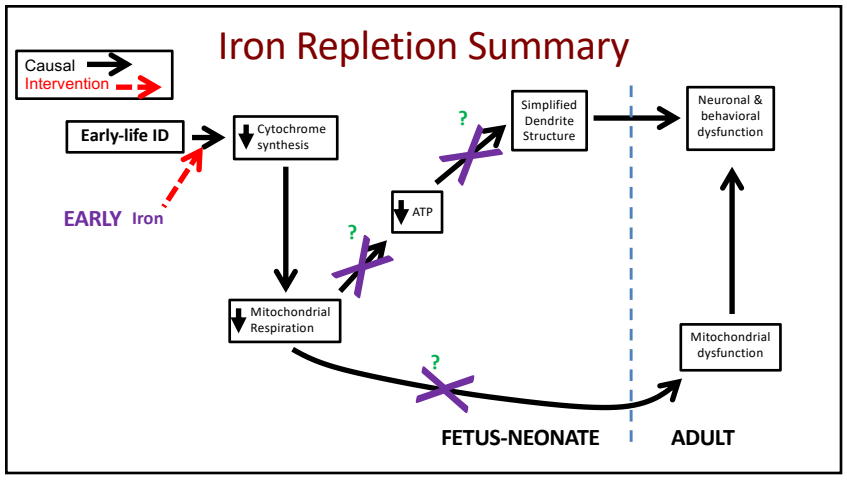


FETUS-NEONATE ADULT


Part 2 – Iron Repletion
Causal
Intervention
Early-life ID
Iron
Cytochrome synthesis
Mitochondrial Respiration
ATP
Simplified Dendrite Structure
Neuronal & behavioral dysfunction
Mitochondrial dysfunction
?
?
?
FETUS-NEONATE
ADULT


Iron Repletion Experimental Design
Third Trimester
Birth
Infant
Toddler
Rapid Hippocampal Development
E16
E19
P3
P7
P10
P14
P17
P21
Dissect hipp, dissociate and plate cells
Begin DFO dosing
Iron Treatment
Iron Treatment
Treatment Groups:
1) Control
2) Iron-Deficient
3) ID + Iron Repletion
DIV
0
3
7
11
14
18
21
25
Establishment of axon and dendrites
Peak dendritic growth and synapse formation
Mature Neuron


Early but not Late Iron Repletion Restores Dendrite Complexity
Rescue 11DIV to 18DIV
Relative ATP Level
1.5
1.0
0.5
0.0
a
b
a
Control
Iron-Deficient
ID + Iron
Rescue 14DIV to 21DIV
Relative ATP Level
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
c
b
Control
Iron-Deficient
ID + Iron
a > b; p < 0.05
Bastian et al., unpublished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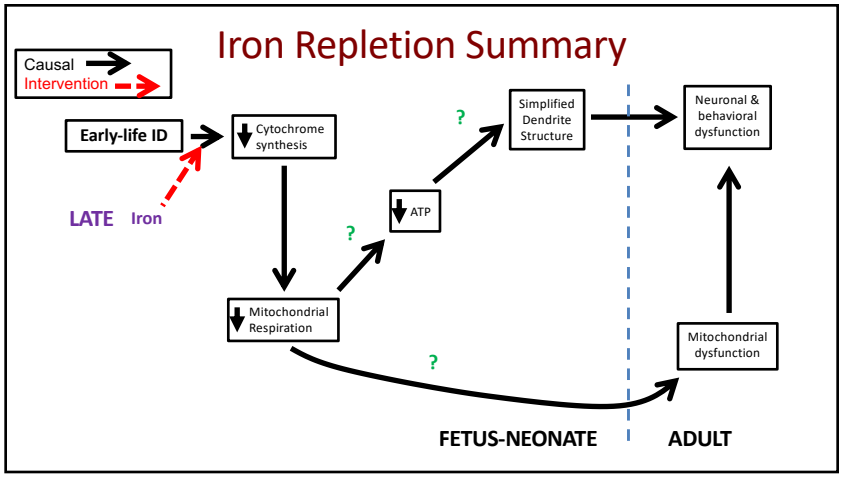

Alternative Treatments for Early-Life Iron Deficiency

- General populations that could benefit:
  1. Those that are not diagnosed with ID early enough and iron repletion will not be sufficient
     - e.g., Diagnosed with ID at birth or one year, after an iron-sensitive period of brain development has passed

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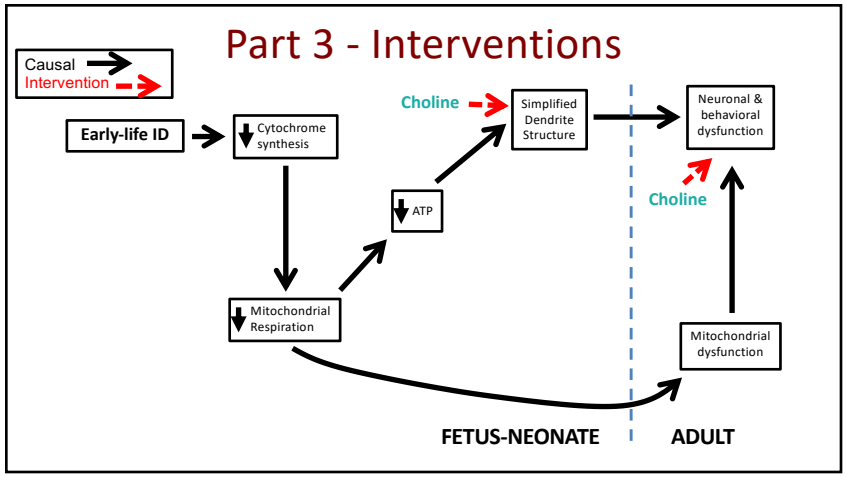
     - e.g., Diagnosed with ID at birth or one year, after an iron-sensitive period of brain development has passed
  2. Those that are diagnosed with ID early enough for iron repletion to be sufficient but they cannot get enough iron
     - e.g., Nutritional ID in low and middle income countries

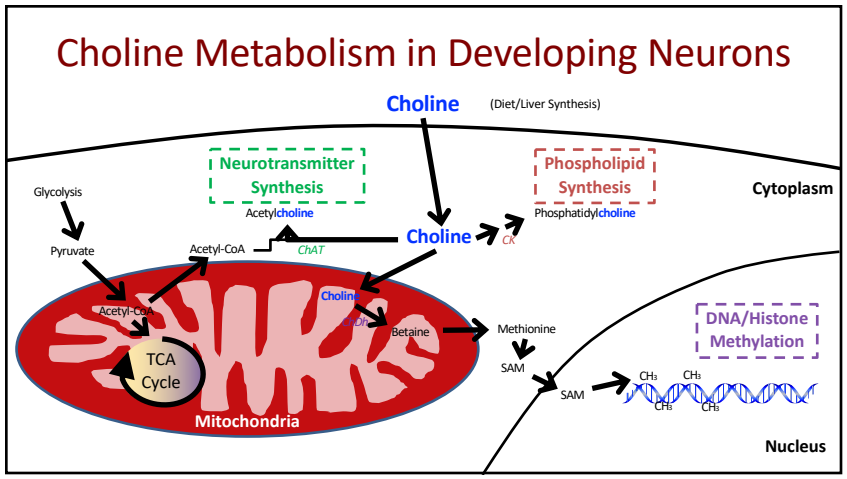
Alternative Treatments for Early-Life Iron Deficiency

- General populations that could benefit:
  1. Those that are <u>not</u> diagnosed with ID early enough and iron repletion will <u>not</u> be sufficient **(Treatment at 14DIV)**
     - e.g., Diagnosed with ID at birth or one year, after an iron-sensitive period of brain development has passed
  2. Those that are diagnosed with ID early enough for iron repletion to be sufficient but they cannot get enough iron **(Treatment at 11DIV)**
     - e.g., Nutritional ID in low and middle income countries



Choline as an Alternative Treatment for Early-Life ID

- Choline is an essential nutrient that is critical for early brain development
- Choline improves learning and memory in animal models of neurodevelopmental disorders:
  - Rett's Syndrome
  - Down's Syndrome
  - Fetal Alcohol Syndrome



Potential Mechanisms of Long-Term Learning and Memory Deficits

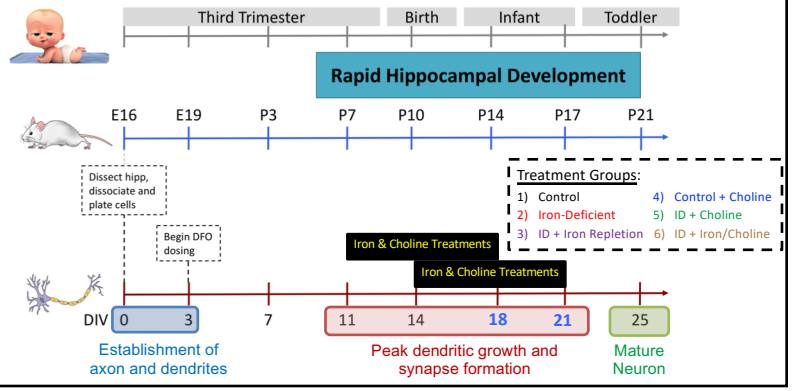
1. Persistent impairment in expression patterns of synaptic genes
   – Epigenetic modifications (e.g., methylation)
2. Residual neuron structural deficits
   – Not enough iron during energy-demanding critical period of hippocampal neuron maturation

Choline

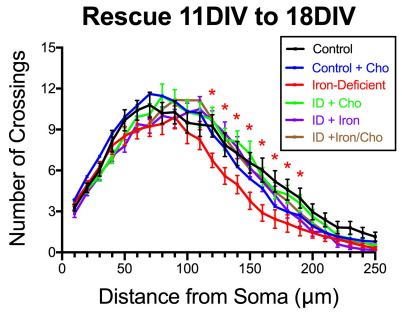
Choline Improves Adult Hippocampal Function after Early-Life ID

- Treatment of iron-deficient pregnant rats with choline during late gestation in conjunction with early postnatal iron repletion improves:
  – Novelty learning/memory (Kennedy et al 2014)
  – Hippocampal gene expression patterns (Tran et al 2016)
  – DNA/Histone methylation at *BdnfIV* promoter (Tran et al 2015)
- **Test whether choline requires iron and whether it can act directly on neurons to improve structure**

Choline Intervention Experimental Design

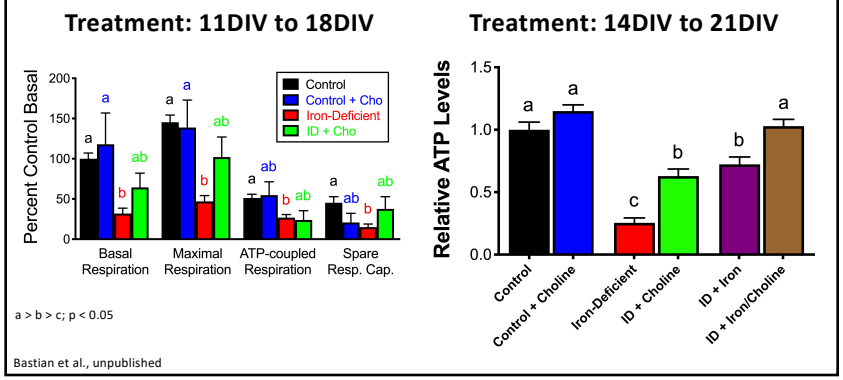


Choline Restores Dendrite Complexity without Iron Repletion



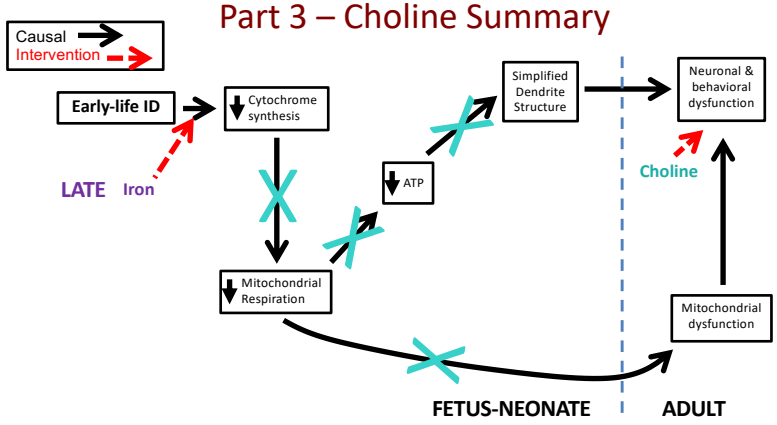
Bastian et al., unpublished

Choline Restores Energy Metabolism without Iron Repletion



Bastian et al., unpublished

Part 3 – Choline Summary



Summary

- Timely iron repletion is sufficient to recover neuronal deficits caused by early-life ID
  - Need for better biomarkers of early-life brain ID
  - Better understanding of iron-dependent critical periods
- Neuronal energy metabolism is a promising therapeutic target for alternative treatment strategies
- Choline may provide a nutritional "work-a-round" to support neuronal development in the face of early-life ID
  - Mitochondria, epigenetics, lipid membranes, neurotransmission
- **Understanding the basic neurobiology reveals novel therapeutic strategies that are not otherwise obvious**

Key Takeaways

- Iron deficiency is still a significant health problem, even in the U.S.
  - Impairs brain development even without causing anemia
  - Maintain iron sufficiency during throughout first 1000 days
- Should we be handing out choline supplements to anyone who might be iron-deficient?
  - No!
  - But…its important that pregnant/nursing women and infants/toddlers are getting enough choline!
- **Building the brain correctly the first time is a lot easier than developing work-arounds**

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1915–2015

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